Diversity of filamentous fungi in organic layers of two forests in Zijin Mountain

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Abstract: A study was conducted to evaluate the cultivable filamentous fungal diversity in organic layers (L, F, and H layers) and A1 layer of two main forest types, *Pinus massoniana* and *Liguidambar formasana* mixed forest and *Quercus variabilis* forest, in Zijin Mountain(32°5' N, 118°48' E), Nanjing, China. A total of 67 taxa comprising 56 Deuteromycetes, 3 Zygomycetes, 5 Ascomycetes and 3 unidentified fungi were recognized from samples from the forest floor of the two forest types. The most abundant group was Deuteromycetes. The dominant genera in both forests were *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Gliocladium* sp. and *Trichoderma* spp. The fungal diversity was higher in the mixed forest than that in *Q. variabilis* forest. For both forest types, the maximum fungal diversity was found in layer F and there existed significantly different in fungal diversity between layer F and layer L. In the mixed forest, richness of fungi isolated from needle litter (*P. massoniana*) was lower than that from leaf litter (*L. formasana*). The richness of fungi from needle litter increased with the increase of forest floor depth, but for leaf litter, the fungal diversity decreased with the depth of forest floor. The co-species of fungi from the two forest types, as well as from two kinds of litters in mixed forest, increased with the depth of the forest floor. The succession of fungi along with the process of decomposition was discussed here. The results also showed that litter quality was a critical factor affecting fungal diversity.

Keywords: Zijin Mountain; Forest type; Filamentous fungi; Diversity; Litter; *Quercus variabilis* forest; *Pinus massoniana* and *Liguidambar formasana* mixed fores

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Introduction

Fungal diversity in organic layers or soils has received increased attention over the last decades (Parungao 2002). This is mainly due to the fact that fungi in soils have great potential for industrial and biotechnological applications (Hawksworth 1990; Lodge 1997). Moreover, the role of fungi in soils on decay of the organic materials is more important than that on the industrial purpose. Twenty functions of fungi were described by Christensen (1989) and one of them was as primary degraders in soils (Paul and Brian 2001). Fungi can grow in and on a wide range of litter and have the unique ability to break down complex substances, such as lignin, cellulose, chitin, keratin and others. Among those organisms, fungi play a leading role in ecosystem maintenance (Subramanian 1982; Rossman 1994). Fungi form a very diverse group and are essential for organic material decomposition and for ecosystem functioning (Heilmann-Clausen and Christensen 2003).

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However, our knowledge of the biodiversity of fungi in forest soils is still poor (Heywood *et al* 1995), especially in China; it is necessary to understand the structure and function of ecosystems in which the fungal diversity plays an important role. It seems that few investigations have been attempted and few studies have been reported in China. The objective of this paper is to investigate the diversity of fungi in different community types and different substrates in a forest.

Materials and methods

Study site

The sampling site is located in Zijin Mountains (32°5' N, 118°48' E), Nanjing, China, with an area of 24 km^2, the peak of 447.1 m, and the altitude of 420 m. The annual mean temperature is 15.4 °C and annual mean precipitation is 1 013 mm. The rainy season comes in June–July. The main forest types of Zijin Mountain are *Quercus variabilis* forest (QvF), *Pinus massoniana /Liguidambar formasana* mixed forest (PLF), and *Q. acutissima* forest (QaF). The QvF and PLF were chosen for this study. The soil type of both QvF and PLF is slight acidic brown soil (pH=5.0). QvF is dominated by *Q. variabilis*, while PLF is dominated by *P. massoniana*, and *L. formasana* (Table 1). The dominated species *Q. variabilis* in QvF is accompanied by the following trees: *Lindera glauca, Dalbergia hupeana, Rhus chinensis, Platycarya strobilacea, Pistacia chinensis, Kalopanax septemlobus, Prunus pseudocerasus, Serissa*

serissoides, Vitex negundo var.cannabifolia, Rubus parvifolius, Rubus corchorifolius, Celtis sinensis, Cinnamomum camphora; and by the herbs including *Corydalis edulis, Trachelospermum jasminoides, Parthenocissus tricuspidata, Carex* sp, *Arthraxon hispidus, Paederia scandens,* and *Smilax china.*

Table1. Characteristics of dominated trees in sampling sites

Species	Height /m	DBH /cm	Density /number·hm^{-2}
Quercus variabilis	24.3	24.1	5.0×10^{2}
Pinus massoniana	23.4	25.6	1.1×10^{3}
Liguidambar formasana	12.5	8.2	5.0×10

For PLF, the dominated *P. massoniana* and *L. formasana* were accompanied by the following trees: *Osmanthus fragrans, Photinia serrulata, Quercus variabilis, Celtis sinensis, Cinnamomum camphora, Lindera glauca, Ligustrum lucidum, Lagerstroemia indica, Kalopanax septemlobus, Firmiana platanifolia, Ilex cornuta, Pistacia chinensis, Ilex purpurea, Symplocos paniculata, Rhus chinensis, Platycarya strobilacea.* The accompanied herbs are *Carex* sp, *Trachelospermum jasminoides, Lysimachia clethroides, Viola verecumda, Parthenocissus tricuspidata, Rubus corchorifolius, Dioscorea sativa, Asparagus cochinchinensis, Arthraxon hispidus, Kalimeris indica, Paederia scandens,* and *Ampelopsis sinica.*

Sampling

One plot (20 m×20 m) was randomly chosen for both QvF and PLF. The plots were further divided into five subplots (4m×5 m). One sample (10 cm×10 cm) was collected from the leaf layer (L), fragment layer (F), humus layer (H), and the ahorizon layer (A1), of each individual subplot. Totally, 40 samples were collected. The samples were kept in a sealed bag and soon taken back to the laboratory of Nanjing University. These samples were stored in refrigerator at 5 °C for further analysis.

Cultivation and identification

Five samples taken from the same layer within the same plot were well mixed. Then mixed samples were subjected to two different treatments. For treatment 1, mixed samples (each weighed 5.0 g) were dried for 24 h at temperature of 60 °C, with air circulation to a constant mass. For treatment 2, mixed samples of layer L, F and H were washed one time with sterilized water, and then sterilized by immersion in 0.1% Hg solution for 1 min, and at last washed three times with sterilized water. The samples were cut into pieces. A 5 g sample was suspended with 45 mL of sterilized water with vigorous shaking for 10 minutes. The original soil suspensions were further diluted with sterilized water to original concentrations of 10^{-1}, 100^{-1}, $1\,000^{-1}$, and $10\,000^{-1}$. A total of 250 μL of these serial diluted soil suspensions were spread on the selective medium containing peptone 5.0 g, glucose 10.0 g, KH_2PO_4 1.0 g, agar 12.0 g, Rhose bengal Sodium Salt 0.003 g, $MgSO_4\cdot7\ H_2O$ 0.5 g, H_2O 1 000 ml. Each dilution has five replicates. The plates were incubated in the dark at 25 °C in a growth incubator. Five days later, the total number of developed fungal colonies was counted. Subsequently, these fungal colonies were identified by colony morphology and the fungi growth. The identification of fungi was based on the references (Zhao *et al.* 2002; Wei 1979; Dai 1979; Deng 1963; Watanabe 1994; Domsch *et al.* 1980). The fungi from the litters of *P. massoniana* and *L. formasana* in the PLF were separately studied. All experiments were conducted in the Lab. of Plant Science, Nanjing University, China.

Data analysis

Richness of fungi was calculated with the formula:

$$N = a \times (u / v) \times (W_F / W_D)$$

where N is colony-forming-unit (CFU)·g^{-1} dry sample, a is the mean of CFU of each plate, u is the dilute times, v is the volume of inoculated spore liquid (250 μl), W_F is the weight of the fresh sample, W_D is the weight of a dried sample. Species frequency was calculated by f=n/N×100%, where f is species frequency, n is a species CFU·g^{-1} dry weight, N is the total species CFU·g^{-1} dry weight.

Results

Diversity of fungi in the organic layers of soils in two forest types

In this study, a total of 67 species comprising of 56 Deuteromycetes (belonging to 49 genera), 3 Zygomycetes (belonging to 3 genera), 5 Ascomycetes (belonging to 4 genera), and 3 unidentified fungi were recognized from the organic layers (layer L, F, H) and layer A1 of the two type of forests in Zijin Mountains (Table 2). The most abundant species was Deuteromycetes fungi followed by Zygomycetes (Table 2). The fungal richness and species identified in PLF were greater than those in QvF, particularly in the case of organic layers. The number of fungal species in QvF was about 70% of that in PLF (Fig.1). The species richness of decomposing fungi occurring organic layers was greater than that of layer A1, especially in PLF. The numbers of fungal species in layer A1 of two forest types were little different.

Diversity of fungi in different organic layers of two forest types

The fungal diversities in organic layers (L, F, and H) and layer A1 of two forest types were surveyed. The result showed that the richness of fungi species in different organic layers of either forest were different. From layer L to A1, the fungal richness increased along the profile in QvF, but decreased in PLF, and diversity of fungi in layer F was the richest (Table 2, Fig.2). Furthermore, it is clear that the species richness and dominant species in each layer were different between the two forest types. Fungal richness in

PLF was higher than that in QvF for all of the three organic layers.

Table 2. Frequency of fungal species in different layers of two forests (10^5cfu·g^{-1}dried sample)

Fungi identified	*Quercus variabilis* forest				*Pinus massoniana* /*Liguidambar formasana* forest						
	Quercus variabilis				*Piuns massoniana*			*Liguidambar formasana*			
	L	F	H	A1	L	F	H	L	F	H	A1
Absidia sp.			6.24	1.70					1.78		
Alternaria sp.	4.68	6.75	6.24	1.70	3.45	6.96	1.01	6.05	3.56		
Aspergillus niger			1.56	6.80	3.45	9.28	9.09	4.84	4.45	4.56	7.62
Aspergillus fumigatus					3.45	4.64	7.07	3.63	5.34	3.42	5.08
Bispora betulina		1.35	1.56								
Botryosporium sp.	1.56					4.64	5.05		3.56	1.14	
Botrytis cinerea			7.8	5.10			4.04		0.89	1.14	
Clasterosporium carpophilum		1.35						2.42	0.89		
Ceratophorum setosum		2.7									
Candida sp.				1.70						5.7	
Cephalosporium acremonium								1.21			
Cephalosporium sp.								3.63	1.78		
Cercospora sp.									0.89	1.14	
Chaetomium bostrychodes	1.56	2.70									
Chaetomium globosum		4.05						6.05	3.56		
Chromosporium sp.			1.56	3.45							1.27
Cladosporium herbarum	6.24	6.75			13.8	6.96	9.09		8.01	13.68	
Coniothecium sp.								1.21			
Cryptostictis sp.				1.70				4.84	2.67		
Dematium sp.				1.70							5.08
Dissophora decumbens								1.21			
Fulvia fulva								1.21	0.89		
Fumago vagans								1.21			
Fusarium sp.	1.56	4.05	6.24			1.16	1.01		4.45	4.56	1.27
Fusarium tricinctum								1.21			
Geotrichum candidum								1.21	0.89		7.62
Gliocladium deliquescens			1.56	10.20	3.45	2.32	1.01	6.05	6.23	10.26	6.35
Graphium penicillioides		1.35		1.70							
Hormiscium sp.		1.35									
Leptostroma hysterioides		1.35									
Mastigosporium sp.				1.70						1.14	
Melanconium sp.		4.05					1.01		5.34		
Monilia sitophila							1.01	2.42	0.89		
Mortierella candelabrum							4.04			5.7	5.08
Mucor sp.	3.12	5.40	7.80	1.70	20.7	8.12	5.05	4.84	4.45	3.42	2.54
Mucor petrinsularis			1.56					1.21		1.14	
Oidium monilioides						8.12	8.08				
Oospora sp.			3.12	1.70							1.27
Ovularia sp.								1.21	0.89		
Paecilomyces varioti		1.35	4.68	8.50		1.16					1.27
Penicillium sp.1	14.04	13.5	10.92	11.90	27.6	12.76	12.12	9.68	8.90	14.82	8.89
Penicillium sp.2		8.10	10.92	6.80		4.64	6.06	12.1	7.12	7.98	13.97
Penicillium sp.3		4.05				6.96	2.02				2.54
Penicillium sp.4						2.32	3.03		1.78	3.42	1.27
Periconia sp.				1.70				4.84	0.89		
Pestalotiopsis sp.	4.68	6.75					1.01	1.21	3.56		
Phoma sp.								1.21			3.81
Phyllosticta sp.									0.89		
Phymatotrichum omnivorum			1.56					2.42			
Rhizopus nigricans		5.40	7.80	5.10		5.8				4.56	
Scopulariopsis brevicaulis		1.35	1.56	5.10				1.21		1.14	6.35
Sclerotium sp.					10.35	4.64					

Continue Table 2

Fungi identified	*Quercus variabilis* forest				*Pinus massoniana* /*Liguidambar formasana* forest						
	Quercus variabilis				*Piuns massoniana*			*Liguidambar formasana*			
	L	F	H	A1	L	F	H	L	F	H	A1
Septoria sp.	1.56										
Sordaria sp.		1.35									
Sphaeronema sp.							1.01				
Sphaeropsis sp.		4.05				1.16	2.02				6.35
Sphaerographium sp.											1.27
Spicaria sp.			4.68	1.70							
Stemphylium sp.							1.01				
Tetracladium sp.											1.27
Trichoderma sp.1	6.24	6.75	6.24	5.10	13.8	3.48	4.04	4.84	6.23	3.42	5.08
Trichoderma sp.2	4.68	2.70	6.24	5.10		4.64	2.02	6.05	5.34	4.56	5.08
Ulocladium zotrycis				1.70				1.21			
Verticillium sp.		1.35		3.40			5.05		3.56	3.42	3.81
Unidentified				1.70					0.89		1.27
Unidentified											1.27
Unidentified				1.70							
Total colonies (10^5cfu · g^{-1}dried sample)	55.30	83.50	91.70	670	25.30	77.10	89.70	71.70	98.50	83.10	103

Note: L: leaf layer; F: fragment layer; H: humus layer; A1: ahorizon layer.

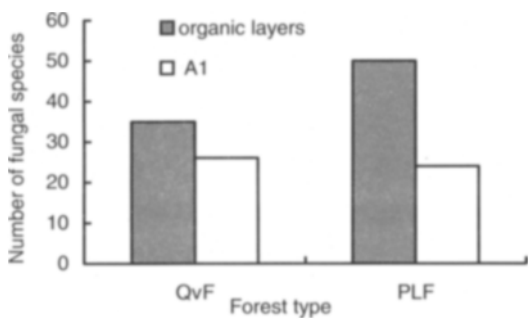

Fig. 1 Comparisons of fungal richness between two main forest types in Zijin Mountain

QvF: *Quercus variabilis* forest; PLF: *Pinus massoniana*/*Liguidambar formasana* forest; A1: ahorizon layer

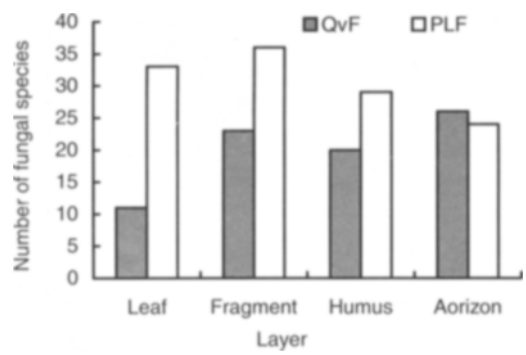

Fig. 2 Comparisons of fungal richness in different layers of two forest types

QvF: *Quercus variabilis* forest; PLF: *Pinus massoniana*/*Liguidambar formasana* forest;

The dominant fungi in each layer were also different. According to the frequency of fungi in each layer of QvF, it is obvious that layer L was dominated (f > 3.12%) by *Alternaria* sp., *Cladosporium* sp., *Mucor* sp., *Penicillium* sp1., and *Pestalotia* sp. Dominant species in layer F (f >4.05%) were *Alternaria* sp., *Chaetomium* sp., *Cladosporium* sp., *Fusarium* sp., *Melanconium* sp., *Mucor* sp., *Penicillium* sp1., *Pestalotiopsis* sp., *Rhizopus* sp. and *Trichoderma* sp. Dominant species in layer H (f > 4.68%) were *Absidia* sp., *Alternaria* sp., *Botrytis* sp, *Fusarium* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium* sp1., *Rhizopus* sp., *Spicaria* sp. and *Trichoderma* sp. Dominant species in layer A1 (f > 5.10%) were *Aspergillus* sp., *Botrytis* sp., *Gliocladium* sp., *Paecilomyces* sp., *Penicillium* sp1., *Rhizopus* sp. and *Trichoderma* sp. (Table 2).

Influence of different litters from a stand on diversity of fungi

Fungal diversity of organic layers between two litters (*P. massoniana* and *L. formasana*) in a stand (PLF) was investigated. It showed that species richness and diversity were different between two litters. From layer L to A1, fungal richness of *Pinus massoniana* was increased. Fungal numbers in layer L and F from *Liguidambar formasana* were greater than that from *Pinus massoniana*. The richness of fungi from *Liguidambar formasana* reached to 31 in the L and F layer, but only 22 fungi was identified in the H layer (Fig. 3).

Based on frequency of each fungal species in each organic layer of *Pinus massoniana*, we found that dominant species in layer L (f >10.35%) were *Cladosporium* sp., *Mucor* sp., *Sclerotium* sp., *Penicillium* sp1. and *Trichoderma* sp. Dominant species in layer F (f > 4.64%) were

Alternaria sp., *Aspergillus* sp. *Botryosporium* sp., *Cladosporium* sp., *Mucor* sp., *Oidium* sp., *Penicillium* sp1., *Rhizopus* sp., *Sclerotium* sp. and *Trichoderma* sp. Dominant species in layer H (f >4.04%) were *Aspergillus* sp., *Botryosporium* sp., *Botrytis* sp., *Cladosporium* sp., *Mortierella* sp., *Oidium* sp., *Penicillium* sp1., *Verticillium* sp. and *Trichoderma* sp. From the litter of *Liguidambar formasana*, we found that dominant fungi in layer L (f> 4.84%) were *Alternaria* sp., *Aspergillus* sp., *Cryptostictis* sp., *Chaetomium* sp., *Gliocladium* sp., *Mucor* sp., *Penicillium* sp1., *Periconia* sp. and *Trichoderma* sp. Dominated species in layer F (f >3.56%) were *Alternaria* sp., *Aspergillus* sp., *Botryosporium* sp., *Chaetomium* sp., *Cladosporium* sp., *Fusarium* sp., *Gliocladium* sp., *Melanconium* sp., *Mucor* sp., *Penicillium* sp1, *Pestalotiopsis* sp., *Trichoderma* sp. and *Verticillium* sp. Dominant species in layer H (f > 3.42%)were *Aspergillus* sp., *Candida* sp., *Cladosporium* sp., *Fusarium* sp., *Gliocladium* sp., *Mortierella* sp., *Mucor* sp., *Penicillium* sp1., *Rhizopus* sp., *Trichoderma* sp. and *Verticillium* sp. Dominant species in layer A1 (f > 3.81%) were *Aspergillus* sp., *Dematium* sp., *Gliocladium* sp., *Mortierella* sp., *Penicillium* sp1., *Phoma* sp., *Sphaeropsis* sp., *Trichoderma* sp., *Verticillium* sp. (Table 2).

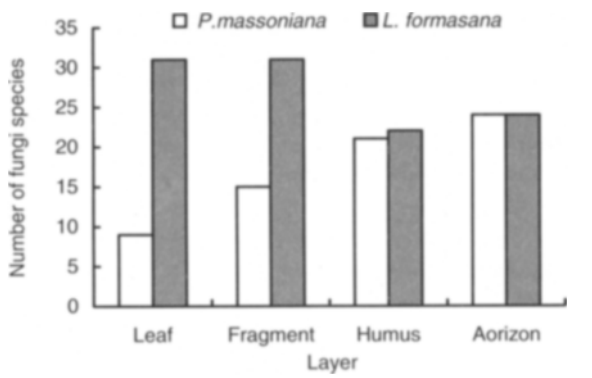

Fig. 3 Changes in fungal richness along with the depth increase of organic layers in the PLF

Discussion

Many fungal species were found in litters in different forest types (Yao *et al.* 1997; Cheng 1993; Christensen M.1989). Fifty-seven taxa comprising of 18 ascomycetes and 39 anamorphic fungi were identified with direct identification method in rain forest (Parungao *et al.* 2002). Seventy fungi were reported in the temperate forest of Japan (Osono 2002). Seventy-seven fungi were found in subalpine coniferous forest of Japan (Ando 1986). In this study, total 67 fungi were found in two forests. The results suggested that the diversity of fungi were varied with different forest and may positively relate with altitude of forest. Number of fungal species from PLF was more than that from the QvF. The reason may be that the higher qualities of litters in the PLF provided more resources for fungi than in QvF. This phenomenon could be even found in different fronds of a plant (i.e. leaves, leaf midribs, petioles, petiole bases) (Hyde *et al* 2000). This indicated that some fungi may preferentially develop in certain tissue types (Huang *et al.* 1998; Zare-Maivan *et al.* 1988). Polishook *et al.* (1996) said that fungal preferences for particular leaf litter probably contributed significantly to the high diversity of fungi in mixed litter that they found in Costa Rica.

The decomposition of plants began with the intrusion of the still growing frond by the fungal pathogens (Osono 2002; Yao *et al.*1997; Hedger *et al.* 1993). After falling down, the leaf litter was then decomposed by a series of fungi. The investigation indicated that the fungi diversity in the same litter layer in PLF was notably higher than that in QvF, suggesting that fungi diversity was related with litter quality. From layer L to A1, the difference of fungal species richness between the two forests was decreased, while the frequency of fungi presented in both forests was increased, reaching at 35% in H layer (Fig.4). This phenomenon was also found from layer L to H between two kinds of litters in the PLF (Fig.5). The reason may be that the diverse litter quality between two kinds of litters or two types of forests decreased with the decomposing process (Tian *et al.* 2002), leading to fungi richness of quality of two litters became similar.

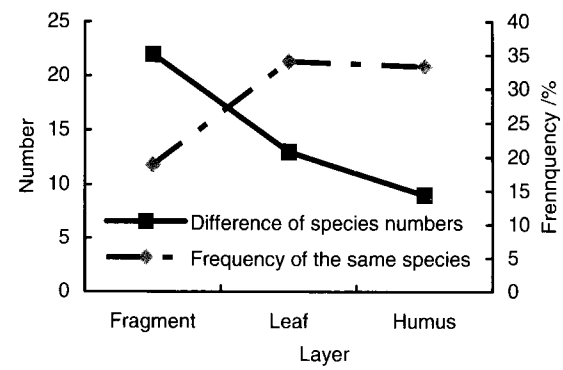

Fig. 4 Changes in richness difference and frequency of common species along profile of forest floor between QvF and PLF

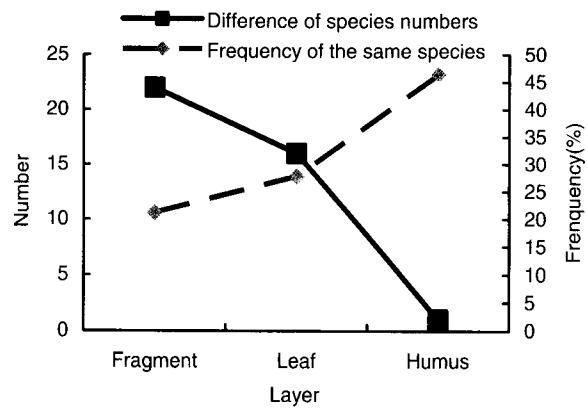

Fig.5 Changes in richness difference and frequency of common species along profile of forest floor between two litters from the PLF

The fungi succession in forest floor correlated with energy and nutrient availability of litter (Zhang 1988). At a micro-scale (on-site) level, average litter depth was a significant factor to affect the occurrence of fungi (Andrew *et al.* 2000). The highest fungi richness occurred in layer F among three organic layers of either PLF or QvF. It showed that there were more fungi activating in layer F than in the other layers. This result was consistent with the work of Heilmann-Clausen and Christensen (2003). Heilmann-Clausen and Christensen (2003) reported that decay stage was found to have the biggest influence on the species richness. Fungal species diversity in logs with decay stages 3 (wood distinctly softened) and 4 (wood highly decayed) was significantly high than that in logs with stages 1 (wood hard) and 5 (very highly decayed). In this study, the richness of fungi in F layer was the highest maybe due to two reasons. The first was that the environmental condition such as temperature, moisture and air conditions in F layer was more suitable for fungi growing than those in the layer L and H (Yao *et al.* 1998). The second reason may be that litter in layer F had higher quality such as nutrients, energy resources as compared with other layers.

It is remarkable that as decomposition processed (layers L to H), the fungi diversity increased in *P. massoniana* forest but decreased in *L. formasana* forest. This is possibly due to the quality difference between two kinds of litters. At the first stage (layers L and F), more retard chemical components and physical structures of litters, such as epidermis, exited in needle than that in broad leaves. Tian *et al.* (1997) reported that more epidermis of needle segments left in the organic layers compared with epidermis of leaves due to its retard structure and chemical components, and only a small number of fungi could successfully colonize and invade in *Pinus massoniana*. In layer F and H, with the destroy of epidermis structure and degradation of holocellulose and lignin and the increase of hard decomposed humus, the difference of chemical components and physical structures decreased, leading to the diversity of dominated fungi of slow decay humus decrease (Wang *et al.* 2001; Yao *et al.* 1997; Yao *et al.* 1998).

Litter decomposition included a series of stages. With decomposition processed, the physical and biological complexity of litters generally increased, leading to an increase of decomposer diversity (Huang *et al.* 1995). A few species of fungi were commonly appeared in whole decomposition stages. Most decomposers were of litter specificity (Li *et al.* 2000). This may be due to the fact that the fungi have their own ecological characteristics to litters. The majority of saprobe fungi can utilize single sugar, starch and cellulose, but few can use holocellulose and lignin (Li *et al.* 2000). Some results showed that the dominant fungi in leaf litter included *Penicillium*, *Aspergillus*, *Cladosporium*, *Herbarum*, *Mucor*, *Trichoderma* *Chaetomium*, and *Fusarium* (Huang *et al.* 1991; Zhang 1988). *Penicillium* and *Aspergillus* played critical roles in degradation of hard decomposing substances (Xu *et al.* 1984; Li *et al.* 1992). Our study showed that the common species in three litter layers in both forest types included *Penicillium* spp., *Trichoderma* spp., *Cladosporium* sp., *Herbarum* sp., *Alternaria* sp.,*Mucor* sp., *Aspergillus* spp. and *Rhizopus nigricans*, suggesting that they can use both single carbohydrates and hard decomposing substances, with wide adaptability. *Pestalotiopsis* sp. *Chaetomium* sp. *Fusarium* sp. and *Melanconium* sp. were special species to break down broad leaf litter, *Sclerotium* sp. and *Oidium monilioides* were special species for needle. These fungi were all in layer F. The species *Absidia*, sp., *Paecilomyces varioti* and *Spicaria* sp. occurred specifically in layer H in *Quercu.acutissima* forest. The species *Oidium monilioides* and *Botryosporium* sp. were special in layer H in *P. massoniana* forest. The fungi *Candida* sp. and *Gliocladium deliquescens* were special species in leaf litter layer H in *L. formasana* forest. Some special species such as *Dematium* sp. and *Phoma* sp. decayed retard substances. The succession of species in litter layers is mainly related to the type of nutrient and the ecological characteristics of fungi. The reasons for the succession may be that host diversity; resource abundance and habitat diversity were different (Lodge *et al.* 1995).

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